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First report of *Hericium cirrhatum* from Pakistan

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Abstract—*Hericium cirrhatum*, a widespread but locally rare tooth fungus is reported for the first time from Pakistan. This species is characterized by white to cream semicircular basidiomata (usually arranged in tiers) with a granular to slightly spiny sterile upper surface, a fertile under surface with 10–15 mm long pointed spines, and ellipsoid to subglobose basidiospores. Morphological characters and DNA sequence analyses inferred from the nuclear ribosomal internal transcribed spacer region (nrITS) confirm the identity of the taxon.

Key words — *Basidiomycota*, *Hericiaceae*, macrofungi, new reports, Swat District

Introduction

Hericium Pers. (*Hericiaceae*) is a saprotrophic genus with worldwide distribution. Macroscopically it is characterized by annual fleshy basidiomata with spiny hymenial surfaces. Important microscopical characters include thick-walled, amyloid, globose to ellipsoid basidiospores, a monomitic hyphal system with clamp-connections, and scattered gloeocystidia (Das & al. 2011). In Pakistan, it is represented by four species: *H. caput-ursi* (Fr.) Banker, *H. clathroides* (Pall.) Pers., *H. coralloides* (Scop.) Pers., and *H. erinaceus* (Bull.) Pers. (Ahmad & al. 1997). During macrofungal surveys in 2015–17 we collected *H. cirrhatum* from two different sites in district Swat, Khyber Pakhtunkhwa, Pakistan. These constitute the first records of *H. cirrhatum* from Pakistan.

District Swat, a floristically blessed land, lies in extreme northern Pakistan in the Malakand division of Khyber Pakhtunkhwa province. It is located at 34°34′–35°55′N 72°08′–72°50′E, covering an estimated area of 5737 km^2. The region is predominantly mountainous with the highest peak of Falak Sar towering almost 6000 m a.s.l. (Barinova & al. 2013). The region is humid, with mild summers, an average annual rainfall >1000 mm, and an 18°C mean annual temperature (Adnan & al. 2006). Annually June (mean maximum temperature = 33°C) is the hottest month and January (mean minimum temperature = –2°C) is the coldest (Barinova & al. 2013). Variations in altitude and exposure greatly modify the climatic conditions within the area. On the basis of altitude, climate, and vegetation, the area can be divided into many climatic and vegetation zones varying from sub-tropical *Pinus roxburghii* Sarg. (chirr pine) forests to alpine pastures and meadows. Forests within the survey area are lush green with humus rich floor supporting large numbers of macrofungi.

Materials and methods

Specimen collection and morphological characterization

Specimens were collected during routine macrofungal forays around Ingaro Dherai village, District Swat, Khyber Pakhtunkhwa, Pakistan and photographed in their natural habitat using Canon Power shot A470 camera; important morphological characters were noted in the field. GPS data were retrieved using a Model 590 Garmin Zumo. The specimens were sun dried, put in zip lock bags and kept in freezer for two weeks to kill any insect larvae. Properly dried collections were deposited in Herbarium, University of Swat, Mingora, Pakistan (SWAT) and in Herbarium, University of the Punjab, Lahore, Pakistan (LAH).

Herbarium specimens were examined microscopically using a BOECO Model BM120 light microscope. After rehydration in distilled water, tissues were mounted in 5% (w/v) KOH solution and stained in 1% Congo red aqueous solution. Melzer's reagent was used to indicate amyloidy of the basidiospores and hyphae. Basidiospore, basidial, and hyphal dimensions were calculated from averages of 20 randomly selected samples from all available basidiomata. Measurements were obtained using Piximètre calibrated software. Values in parentheses represent extremes, Q = length/breadth ratio of individual spores, Qe = average length/breadth ratio of all spores from all basidiomata, and Me = average spore measurements. Microscopical characters were drawn using a camera Lucida.

Molecular characterization

DNA extraction followed Porebski & al. (1997). The universal primer pair ITS1F and ITS 4 (White & al. 1990; Gardes & Bruns 1993) was used to amplify the nrDNA Internal Transcribed Spacer region (ITS) comprising ITS1, 5.8S, and ITS2 following

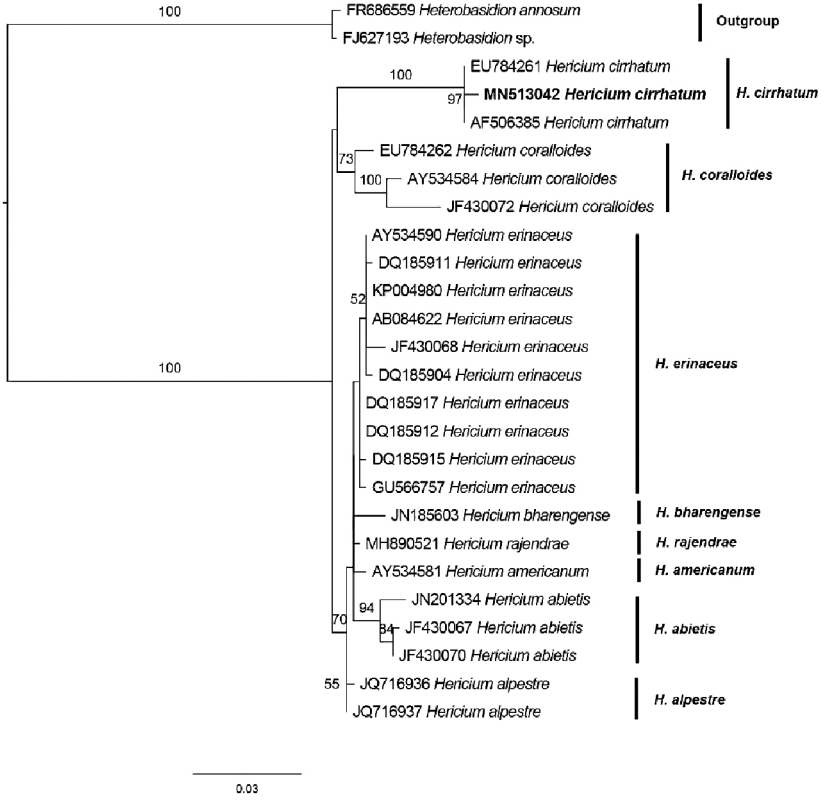

Fig. 1. nrITS based maximum likelihood analysis of *Hericium cirrhatum* and related taxa using RAXML-VI-HPC. Pakistani collection is presented in bold. Bootstrap values ≥50% are given above nodes.

Gardes & Bruns (1993). The PCR amplified product was sequenced by Beijing Genomic Institute (Hong Kong).

The consensus sequence was generated from both forward and reverse primer reads using BioEdit (Hall 1999) and compared with other sequences in the GenBank using the Basic Local Alignment Search Tool (BLAST) on the NCBI website. Closely matching unpublished and published sequences (Das & al. 2011, Hallenberg & al. 2013, Singh & Das 2019) were downloaded for phylogenetic analysis. *Heterobasidion annosum* (FR686559) and *Heterobasidion* sp. (FJ627193) were selected as outgroup taxa following Hallenberg & al. (2013). The downloaded sequences were aligned using Multiple Sequence Alignment Search tool (Muscle) and Clustal X2 (Larkin & al. 2007).

Maximum Likelihood analyses were performed using CIPRES and run in RAXML-VI-HPC (Stamatakis 2006) using the GTRCAT model. Branch support was calculated by 1000 bootstrap replicates.

Phylogenetic analysis

BLAST analysis of the 572 bp ING-26 consensus sequence (Fig 1) showed 99% similarity with *Creolophus cirrhatus* (Pers.) P. Karst. [≡*Hericium cirrhatum*] GenBank EU784261 (from Britain) and AF506385 (from Sweden).

The 26 nucleotide sequences used for phylogenetic analysis represented eight ingroup taxa and two outgroup taxa. After trimming and aligning, the final dataset comprised 548 total sites, of which 142 were variable and 109 parsimony informative. Maximum likelihood analysis nested the Pakistani collection with *Hericium cirrhatum* from Britain and Sweden, confirming its identity as *H. cirrhatum*.

Taxonomy

Hericium cirrhatum (Pers.) Nikol., Acta Inst. Bot. Acad. Sci. USSR Plant. Crypt., Ser. 2, 6: 343. 1950. Figs 2, 3

Basidioma 70–100 mm across, shelf-like, mostly in lateral tiers attached at the base, semicircular to irregular, soft to touch when fresh, hardening upon drying, pure white at first, then turning cream colored, and finally drying brown. Upper surface granulose to echinulate with small sterile spines; margin thin and spinose. Under surface spiny; spines 10–15 mm long, pointed with tapering ends, close, white to creamy at first then turning pinkish and finally brownish on drying. Context concolorous to the exterior, dry to moist, fibrous, 20–30 mm thick at the center and 5–15 mm at the edge. Stipe absent.

Basidiospores (3–)3.1–3.7(–3.9) × (2.2–)2.3–2.9(–3.2) µm, Q = 1.2–1.4, Me = 3.4 × 2.7 µm, Qe = 1.3, broadly ellipsoid to ellipsoid in side view, subglobose to ovoid in face view, smooth, with apiculus, amyloid, hyaline in KOH. Basidia tetrasporic, 30–40 × 4–6 µm, slender, smooth, basal clamp connections present, hyaline in KOH. Contextual hyphae ≤5 µm in diameter, thick-walled, with clamp-connections. Gloeoplerous hyphae 4–9 µm in diameter, frequent, present in both trama and context. Gloeocystidia 75–110 × 6–9 µm, scattered, sinuous.

Material Examined: **PAKISTAN**, **Khyber Pakhtunkhwa**, **Swat district**, Ingaro Dherai, village 34°47′19″N 72°19′28″E, 920 m a.s.l., saprobic on decomposing *Populus*

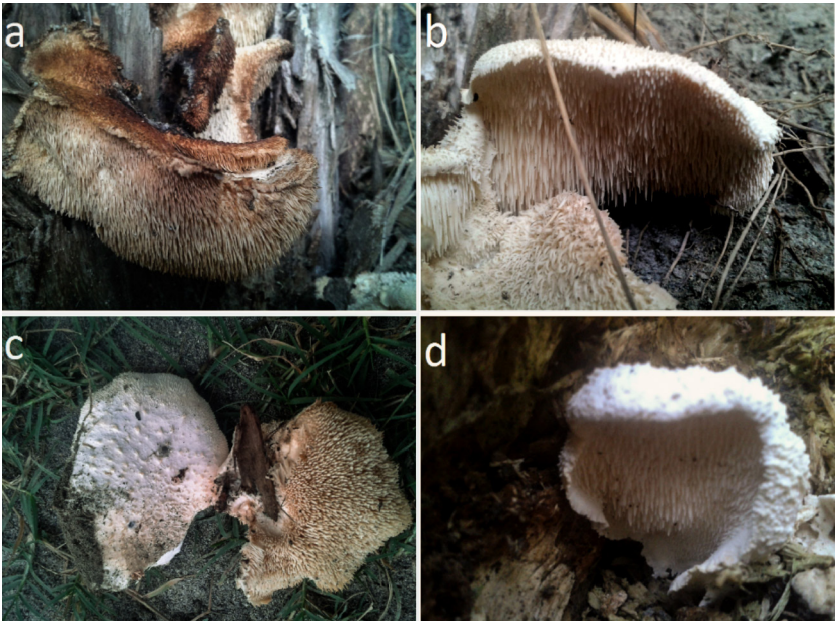

Fig. 2. *Hericium cirrhatum* basidiomata, showing different collections and stages of development. a–c. ING-26 (SWAT001359); d. ING-33 (SWAT001364).

stump, 30 April 2015, Junaid Khan ING-26 (SWAT001359; GenBank MN513042); Ingaro Dherai, 34°47′11″N 72°19′37″E, 925 m a.s.l., in decaying cavities of standing *Populus* tree, 7 August 2015, Junaid Khan ING-33 (SWAT001364).

Discussion

Hericium species are generally rare and grow on wood of broadleaved trees (Boddy & al. 2011). Among the different *Hericium* species, *H. cirrhatum* is rarely documented, and the species has been considered very rare in Britain (Evans & al. 2007, Reilly 2011), Germany, Russia (Volobuev 2013), and most of Europe (Parfitt & al. 2005). In Asia, *H. cirrhatum* has been reported from India (Das & Sharma 2009) and Japan (Crockatt 2008), with no previous reports of this species from Pakistan (Ahmad & al. 1997). Our collections represent a new record for Pakistani mycoflora.

Hericium cirrhatum commonly grows on fallen or cut trunks and branches, and damaged parts of standing trees of *Fagus sylvatica* L., *Quercus* spp., *Acer* spp., *Betula* spp. (Boddy & al. 2011), *Populus tremula*

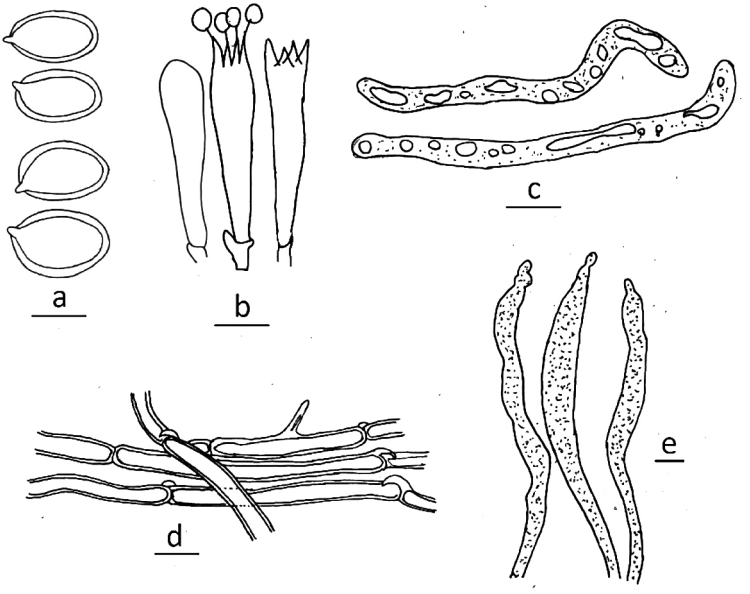

Fig. 3. *Hericium cirrhatum* (SWAT001359). a. Basidiospores; b. Basidia; c. Gloeoplerous hyphae; d. Contextual hyphae; e. Gloeocystidia. Scale bar: a = 2 μm; b–e = 8 μm.

L. (Volobuev 2013), and *Alnus nepalensis* D. Don (Das & Sharma 2009). In our study area, all samples were growing on decomposing stumps or damaged parts of standing *Populus* trees, which is in accordance with the Russian collections.

Morphologically, the present Pakistani collections agree well with the description of *H. cirrhatum* by Das & Sharma (2009). *Hericium cirrhatum* most closely resembles *H. erinaceus*, which is distinguished by longer and more compact spines and more or less circular and fleshier basidiomata. *Hericium caput-ursi,* another closely related species can be distinguished by a branched basidioma with spines hanging in tufts from branch tips.

Distribution data of globally rare taxa are useful, especially from a conservation standpoint. New data calibrate the criteria available for red data listing and help reassess the status of some species (Boddy & al. 2011).

The present report extends the global distribution of *Hericium cirrhatum*, but further studies are recommended to refine the distribution range of this species within Pakistan and in adjoining countries.

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